

A Look at Cirri Presence and Preference: Utilizing Field and Laboratory Studies to Explore a
Novel Appendage in *Hippocampus zosterae*

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ABSTRACT

Dwarf seahorses (*Hippocampus zosterae*) are a cryptic species displaying filamentous appendages, known as cirri, which have been historically assumed to have evolved for camouflage purposes. To investigate potential patterns in cirri presence in *H. zosterae*, photos from monthly sampling over a year-long period in Tampa Bay, Florida were analyzed to deduce which environmental parameters could be correlated with cirri. Cirri presence on wild caught seahorses was quantified using a scoring system of cirri on three head areas and one body area that summed to an overall cirri score for each fish. Preliminary results indicated that females had more cirri than males, and that fish with more cirri were present during the wet season, coinciding with the breeding season of *H. zosterae*. The sexual dimorphism identified in this trait for dwarf seahorses led to a hypothesis centered around cirri's potential role in mating preferences. To determine if cirri are a sexually selected trait, wild seahorses were brought into the laboratory for mating trials consisting of three size-matched seahorses: a male, a highly ornamented female, and a less ornamented female. We developed a novel methodology for photographing female seahorses to measure cirri counts, length, and surface area and documented the potential loss of cirri in a captive setting. Our genetic results confirmed the highly ornamented female was chosen in 17 out of 19 trials, supporting our hypothesis that this trait serves as a sexually selected trait with males showing a mating preference for females with greater cirri. Drastic cirri loss was recorded for females over 21 days in captivity, prompting new hypotheses that cirri function as a condition-dependent trait involved in mate signaling, and may serve as an indicator of seahorse health and survival.

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Chapter I: Introduction

Natural and sexual selection are fundamental concepts in ecology that are often intertwined. Natural selection favors traits that enhance an organism's survival in their environment, while sexual selection favors traits that give an organism an advantage when competing for or attracting a mate (Darwin, 1859, 1872). Sexual selection often leads to sexual dimorphism in species, meaning that the males and females are phenotypically different (Darwin, 1872). One aspect where these two selective pressures overlap is through condition-dependent traits. Condition-dependent traits are observable features that become more prominent based upon the health or condition of the organism (Hill, 2011). These traits are also commonly referred to as phenotypically plastic, as the degree to which the traits are displayed can change based on the surrounding environment (Sommer, 2020). A classic example of this phenomenon occurs in the species *Poecilia reticulata* (Guppy). Male guppies can display elaborate coloration that is a result of their diet containing carotenoids from food sources, primarily from benthic algae in their environment (Karino & Haijima, 2004). Males who are better foragers are likely to be more colorful, but the degree of their coloration is dependent on how much food they obtain (Karino & Haijima, 2004). While male guppy coloration is a direct mechanism of their diet, the level to which they display their coloration is also influenced by predator presence (Price et al., 2008). In areas where guppies co-exist with known predators, the males are much drabber, as they must channel more energy into survival and reproduction instead of their coloration (Endler, 1980). Male guppies found in habitats without prominent predation threats are much more colorful because they can dedicate more energy and effort into foraging (Endler, 1980). Guppies

are also a great example of how condition-dependent traits are often a result of balancing natural and sexual selection pressures. Males are selected to be colorful to attract a mate, the influence of sexual selection, but not so colorful that they can easily be spotted by a predator, the pressure of natural selection (Endler, 1987). A family of organisms that frequently displays condition-dependent traits in an interesting manner, as they are often exhibited by the females due to sex-role-reversal, is the family Syngnathidae (Rosenqvist & Berglund, 2011).

The family Syngnathidae is a broad group of iconic fishes, including seahorses, pipefishes, and seadragons, that share unique morphologies and life history traits, with paternal brood care as the most well-known feature of these fishes (Mobley et al., 2011; Rose et al., 2019). A prominent phenotypic trait across some of the species in this family, as well as other marine animals, are ornamented, sporadic appendages known as cirri (Brown et al., 2020; Qu et al., 2023; Woodall et al., 2018). The dwarf seahorse, *Hippocampus zosterae* (Jordan & Gilbert, 1882), like other species of seahorses, display cirri in the form of branched, stick or hair-like filaments that can be found on their body and head (Woodall et al., 2018).

Dwarf seahorses are one of the smallest of over forty-six currently recognized species of seahorses, reaching sexual maturity at a length of around 25 mm (Lourie et al., 2016; Rose et al., 2019; Short et al., 2020). Their known range includes seagrass beds from Bermuda to the Bahamas, along the Florida Atlantic coast, and into the Gulf of Mexico (Chaplin, 1968; Ginsburg, 1937; National Marine Fisheries Service, 2020). This species exhibits both social and genetic monogamy, with evidence of a male and female pair bonding repeatedly throughout a breeding season (Masonjones & Lewis, 1996; Rose et al., 2014). Despite their monogamous mating system, which would generally assume an even sex-ratio in a population, the dwarf seahorse populations in Tampa Bay, Florida exhibit a female-biased sex ratio that is most evident

in the late summer-autumn to winter season (Rose et al., 2019). Gravid males appear in the highest proportions during this time, suggesting that the peak breeding season for dwarf seahorses may occur during the months of August to October (Rose et al., 2019). *H. zosterae* densities are highest in Tampa Bay around the same time, as this peak breeding season coincides with the wet season in Tampa (May – October) (Masonjones et al., 2010). Like other species of seahorses, *H. zosterae* are poor swimmers with low mobility and high site fidelity (Fedrizzi et al., 2015). Due to their limited mobility, seahorses often use their tail to cling to items in their surrounding environment, which are known as holdfasts (Woodall et al., 2018). The most common holdfast for seahorses is seagrasses, and seahorses can change their color over time to camouflage with their surroundings while immobile in their environment (Foster & Vincent, 2004).

H. zosterae makes for a good model system for this thesis as they are a well-studied species, can be maintained in captivity for breeding studies, and abundant in our sample collection site in Tampa Bay, Florida. Multiple population studies have been conducted in Tampa over the last twenty years, with each study confirming the stability and abundant numbers of *H. zosterae* in the wild (Masonjones et al., 2010; Masonjones et al., 2019; Rose et al., 2019). They have been successfully maintained and bred in captivity, making this a good species for laboratory studies (Masonjones & Lewis, 2000; Masonjones & Rose, 2019). Males have assured paternity due to their specialized brood pouches with internally fertilized and incubated eggs which results in live birth of offspring, creating an ideal system for preference mating trials and subsequent parentage analysis (Masonjones & Lewis, 1996; Rose et al., 2019). Dwarf seahorses also serve as an indicator species for the seagrass beds they inhabit, making them an important population to monitor for understanding the overall health of these critical seagrass ecosystems

(Shokri et al., 2009). Despite their importance to these coastal habitats, dwarf seahorse populations are threatened by numerous anthropogenic activities, such as dredging, coastal development, and commercial harvesting (Fedrizzi et al., 2015). Additionally, seagrass beds are a highly fluctuating environment, with the beds in Tampa having experienced dramatic declines and rebounds in the past (Sherwood et al., 2017). Gaining a deeper understanding about the biology of this species, especially an unknown feature like cirri that may reflect environmental quality, could be important for future conservation purposes.

The term cirri and other hair-like structures are found throughout the animal kingdom. Barnacles use cirri to capture food, as they are important mobile appendages for otherwise sessile creatures (Hindenberg et al., 2022). Other hair and hair-like structures, which include a diverse range of appendages from whiskers in cats (*Felis catus*) to cerci in crickets (*Acheta domesticus*), can be used for foraging, coordinated movement, and collecting sensory information (Boublil et al., 2021). Although no proper studies have been conducted regarding the role of cirri in seahorses, some basic observations in other species beyond *H. zosterae* have been reported that could allude to three possible functions for these appendages: the historical assumption of camouflage, potential as a nutritional reserve, and a sexually selected trait used in mate choice preferences (Correia et al., 2018; Lourie et al., 2004).

Cirri are usually classified as another aspect of a seahorse's camouflage, a fair deduction when considering the environment in which seahorses reside (Curtis et al., 2017). The hair or stick-like appearance of cirri closely resembles the epiphytes or macroalgae that can be found within the seagrass beds as seahorses (Brodersen & Kühl, 2022; Woodall et al., 2018). Cirri are also capable of physiological color change, containing branched melanophores that could aid in the movement of melanosomes, allowing seahorses to further color-match with their

environment (Gristina et al., 2017). An interesting pattern observed in *Hippocampus whitei* is the presence of cirri on juvenile seahorses, but a lack of cirri on adults (Melhuish, 2025).

Additionally, laboratory reared juveniles in this species often do not develop cirri, a trend that is also seen in juvenile *H. zosterae* raised in captivity (Melhuish, 2025; Sanchez, 2025). This could indicate a necessity for some form of signaling from a wild environment to induce cirri development, such as cues in the water column from nearby predators or dense macroalgae.

Without these cues, juveniles may lack the unknown cascade of internal signaling that might be required to onset the development for this trait or not have a physiological need to develop cirri that would help them camouflage with their environment.

A second potential function of cirri, for mating preference, stems from a pattern observed in a European species of seahorse, *Hippocampus hippocampus*, or the short-snouted seahorse. The females in this species display more cirri than males, which contrasts from *Hippocampus guttulatus*, where there is no difference in cirri between sexes (Woodall et al., 2018). Cirri have been reported to be a predictable feature of sexual maturity in *H. guttulatus* though, with the development of cirri coinciding with the development of a pouch or ovaries (Curtis, 2006). Females having more cirri than males in *H. hippocampus* could indicate cirri as a sexually dimorphic feature in this species, and possibly other species of seahorse as well. Sexual dimorphism in traits related to mating is common in Syngnathidae species. In several pipefish species, female fishes display some form of coloration or patterning, especially during the breeding season, that the males either lack or do not display as prominently (Flanagan et al., 2014; Mobley et al., 2018; Monteiro et al., 2017). For example, both males and females in *Nerophis lumbriciformis* exhibit a spotted facial coloration, but the females' coloration is much more distinct (Monteiro et al., 2017). Dramatized coloration has direct links to reproductive

benefits in this species, as females with more coloration mate more often, have larger eggs, and higher gonadosomatic index values (Monteiro et al., 2017). The facial coloration is a plastic, condition-dependent trait though, as females only invest substantial energy into their facial coloration when exposed to breeding season temperature water (Monteiro et al., 2017). Like coloration in pipefish, cirri could serve as an ornamental feature in seahorses for which males display a preference.

Whether as a form of camouflage or as a preferred trait during mate choice, cirri serve an unknown purpose in seahorses and appear to be a condition-dependent trait. As selection pressures often influence condition-dependent traits, cirri could potentially be under multiple forms of selection. Natural selection might be driving cirri presence to allow individuals to avoid detection from predators, while sexual selection pressures lead to increased cirri presence for attracting mates. The apparent need for environmental signaling or habitat complexity to trigger the development of cirri in some species of seahorses provides ample evidence for this hypothesis, but to our knowledge, no formal studies have been conducted to determine what environmental patterns could predict cirri presence, nor the possible functions of this feature in seahorses. Using *H. zosteræ* as our model system, the following thesis aims to (1) establish novel protocols for cirri quantification in both a field and laboratory setting. Using the field protocol, we will (2) investigate patterns of cirri in wild-caught seahorses and which varying environmental factors may impact cirri presence. Based on the results of our field study, we conducted mating trials in the laboratory to determine if cirri are a sexually selected trait to (3) test our hypothesis of a potential mating preference function of cirri. Lastly, we wanted to determine if cirri showed condition dependence by (4) tracking and quantifying cirri presence over time in captive females with laboratory quantification protocols.

Chapter II: Materials and Methods – Wild-Caught Studies

Year-long Sampling Events

Monthly sampling was conducted at two different sites, a north and south site within a continuous seagrass bed in Tampa Bay, Florida from August 2022 to July 2023 (Figure 1). Both sites were located within a shallow seagrass bed with 100% *Thalassia testudinum* cover, and sampling was performed in predetermined concentric circular plots divided into 4 rings and 18 transects as described in Sims (2018) (Figure 2). A pole was placed in the middle of the circles where a person would stand to orient direction and guide two people using a 1.5m push net. The push net was modified with zip ties and attached to the pole by a rope to control distance from the center (Masonjones et al., 2010; Sims, 2018; Strawn, 1958) This modified net was used to gently comb through the seagrass along each transect starting in Ring 4, the outermost ring, and pulled in a clockwise fashion (Masonjones et al., 2010; Sims, 2018; Strawn, 1958) After a ring was finished, the net would be moved inward to the next ring. This methodology was used to collect primarily *H. zosterae* and *Sygnathus scovelli*, and any caught were placed into buckets labeled for each sampled transect when a continuous net pull occurred. Collected fish were then brought back to the University of Tampa's Marine Science Field Station. Each seahorse was placed in a tank next to a metric scale to have photographs for the seahorse's full body and a close-up of their head taken of the fish's left side. While the fish were being photographed, the number of males, females, and juveniles collected in each transect was documented. After cataloging the fish, they were returned to the center of the sites where they were collected. Fish

were collected under Florida Fish and Wildlife’s SAL-22-2319-SR and handled according to Valdosta State University’s AUP-00081-2021 (Appendix A). Various abiotic factors were recorded during each sampling event, including season (wet or dry), water temperature (°C), salinity (ppt), and turbidity (ntu), as well as biotic factors such as macroalgae density (g/m²) and seagrass cover as stated in Sims (2018).

Figure 1

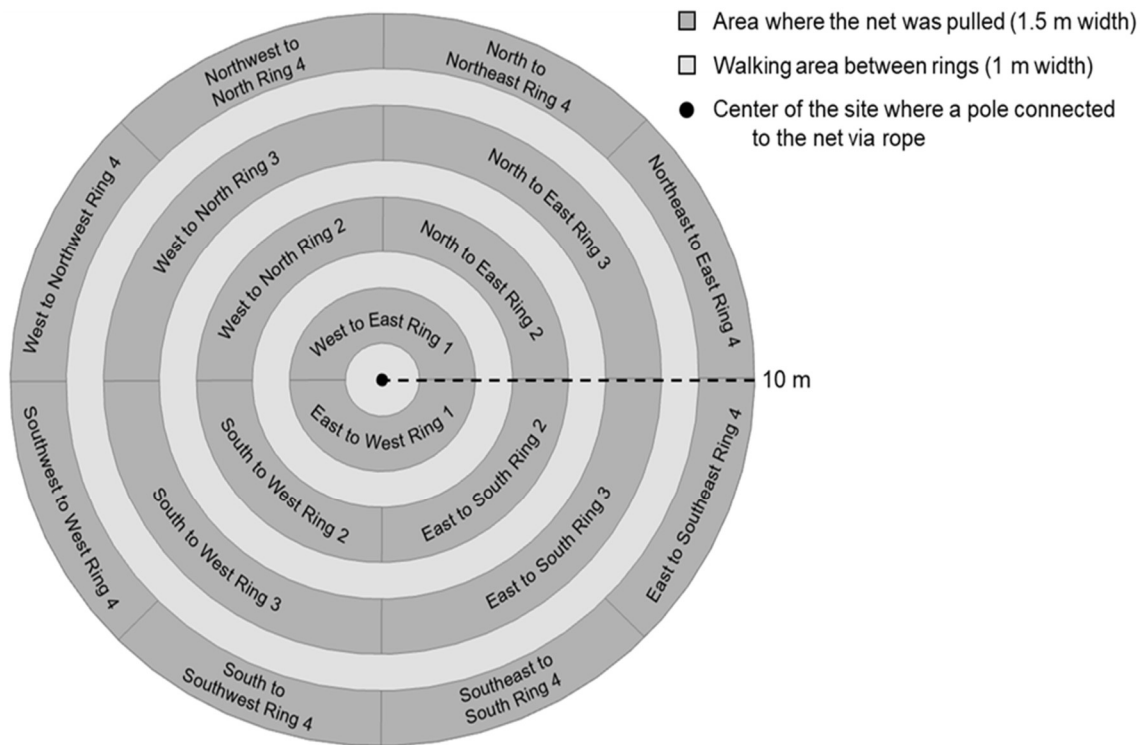
Images of year-long study sampling locations



The North Site (white triangle) and South Site (white square) are located within a continuous seagrass bed in Tampa Bay and utilized for monthly sampling events. Adapted from Sims, 2018.

Figure 2

Diagram of the concentric circular plot located at each site



Each plot had a total radius of 10 m. The outermost Ring 4 was divided into 8 transects that were 10.90 m², Ring 3 was broken into 4 transects of 15.9 m², Ring 2 was broken into 4 transects of 10.02 m², and Ring 1 was broken into 2 transects of 8.25 m². Adapted from Sims, 2018.

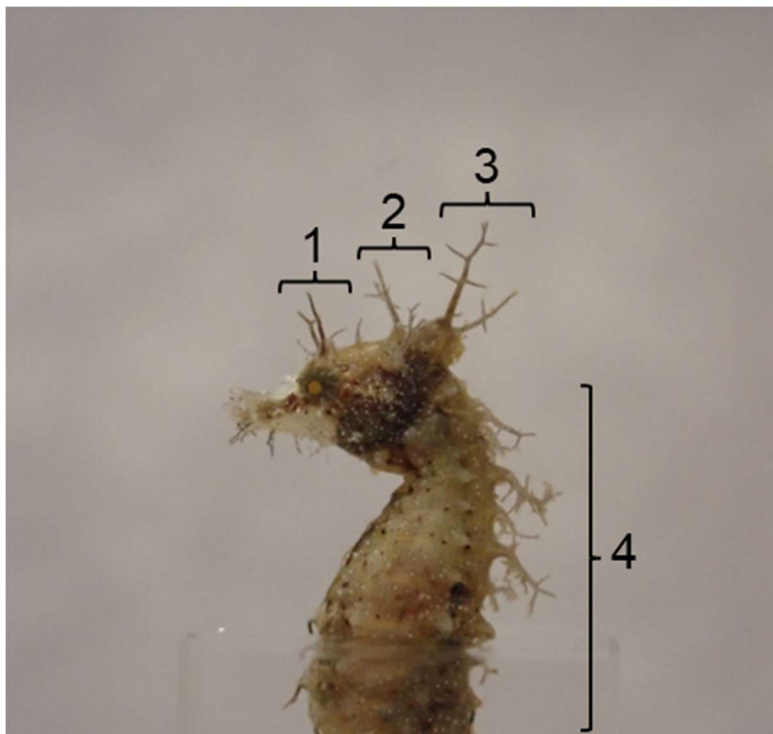
Wild-Caught Cirri Measurements

Photos of seahorses caught in Tampa Bay during the year-long sampling study were assessed for cirri presence on their head and body using the cirri counting protocol we developed for our field photographs. Body cirri were grouped into one general area, and the head cirri were divided into three sections: above the eye, at the center of the head, and on the crown of the head (Figure 3). For the head cirri, each area was given either a 0, 0.5, or 1 to represent absent, immature, or present cirri, respectively, in the area, while the body cirri were given either a 1 or a 0 for presence or absence if the seahorse had any cirri along its body. An estimate of the

diameter of the seahorse's eye was used to differentiate between 0.5 or a 1, with a 1 being longer than the diameter. A total head cirri score was calculated for each seahorse by adding the numbers given to the three head areas together, as well as a total cirri score where the body cirri number was included.

Figure 3

Four areas assessed for cirri presence in dwarf seahorses



Cirri were assessed with a score of 1, 0.5, or 0 to account for present, reduced, or absent cirri, respectively, at four locations: (1) Eye cirri, (2) Center cirri, (3) Crown cirri, and (4) Body cirri. The eye diameter was used as a reference point to differentiate between a 0.5 and a 1. In this example, each area would be given a 1, as all cirri are easily visible and larger than the eye's diameter. This gives a total head cirri score of 3, and a total cirri score of 4.

Statistical Analysis

Influences of Group and Season on Total Head Cirri

All statistical analyses for this thesis were performed in R (version 4.5.1, R Core Team, 2025). To clarify for the statistical test outlined below and following statistics, the term “group” will refer to males, females, and juveniles, while “sex” will refer to sexually mature males and females. For statistical analyses of wild-caught seahorses, the total head cirri score was used instead of the total cirri score. Since the head cirri are divided up into more areas than the body cirri, the head cirri score is a more precise measurement of cirri presence until more detailed body areas are designated. After running a Shapiro-Wilk normality test, the response variable of total head cirri was determined to be non-parametric. The effects of group (female, male, and juvenile) and seasonality (wet or dry), as well as the interaction between the two, on total head cirri of all seahorses were determined using an aligned rank transform (ART) ANOVA implemented using the R package ARTool (Wobbrock et al., 2011; Ye et al., 2023). The pairwise comparisons for group and season were tested using the emmeans function implemented in the emmeans: Estimated Marginal Means, aka Least-Squares Means R package (Lenth & Piaskowski, 2025). After running this test, juveniles were removed from the dataset, except for density calculations, to more closely examine cirri as a sexual signal for adults in the rest of the analyses. Sex and seasonality were included in the following analyses because these factors appear to be strong determinants of cirri presence, even though the tests will focus on other factors.

Abiotic Water Quality Effects on Total Head Cirri

A generalized linear model (GLM) was used to investigate the effects of temperature, salinity, turbidity, sex, and season, on total head cirri. The largest possible model was analyzed

with the dredge function implemented in the MuMin R package, and based upon the lowest AICc values, a model that included temperature, salinity, sex, season, and an interaction between salinity and temperature was deemed to be the best fit model (version 1.48.11, Bartoń, 2025). This model excluded turbidity, but the effects of turbidity had been deemed insignificant in preliminary analyses prior to running the dredge analysis. The Gamma family and log link were used for this model.

Biotic Water Quality Effects on Total Head Cirri

A GLM was conducted to analyze the effects of macroalgae density, sex, and season, on total head cirri. After confirmation using the dredge function, a model including all three factors was conducted using the gaussian family and the identity link. Macroalgae densities were calculated as the dried weights over the area for each transect using six representative handfuls of macroalgae (Sims, 2018). August is excluded from this model due to a lack of data for individual rings and transects on that date.

Effects of Population Factors on Total Head Cirri

A GLM was used to explore the effects of density, sex ratio, sex, season, and the interactions between them on total head cirri. Density was calculated as the total number of adults and juvenile seahorses over the area sampled by the net for each transect, and sex ratio was the number of males to the total number of adults caught in each transect. Like the last model, August is also excluded due to a lack of data about which transect and ring seahorses were caught within. After confirmation with the dredge function using the largest possible model, the model with the lowest AICc value included density, season, and sex. A preliminary GLM was conducted that included sex ratio, which was determined to be nonsignificant, so sex ratio was removed. This model was conducted with the gaussian family and identity link.

Chapter III: Materials and Methods – Laboratory Studies

Sampling for Captive Studies

Sampling for laboratory studies was conducted in the same seagrass beds as the wild-caught 2022-2023 surveys in Tampa, Florida on June 3-4, 2024. A modified push net method was used within the beds to collect *H. zosterae* adults (Sims, 2018). All fish were collected and handled under Florida Fish and Wildlife's SAL-22-2319-SR and Valdosta State University's AUP-00086-2024 (Appendix B).

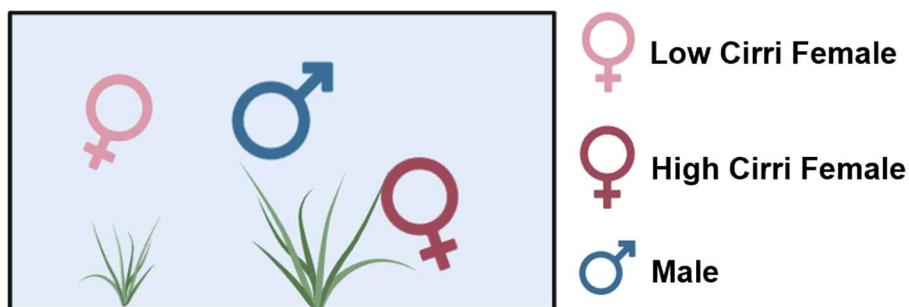
Mate Preference Trials

The collected seahorses were transported from Tampa to Valdosta, Georgia and housed in Valdosta State University's Aquatic Laboratory for 21 days. Seahorses were kept in salt water around 32 ppt, which matched the salinity of Tampa Bay on sampling days, and at a room temperature of 24 °C. Female seahorses were kept in individual 2.5-gallon tanks, and to avoid the potential influence of outside visual or chemical signaling between fishes on cirri presence, all sides of the tank that faced other females were occluded. The males were first categorized by pregnant or non-pregnant, sorted by their stage of pregnancy, and then grouped in 5- or 10-gallon tanks. Brine shrimp (*Artemia salina*) were hatched and fed to the seahorses every day. Water quality was tested every 1-2 days, and frequent water changes occurred throughout this time to maintain water quality conditions. The room was kept on a 12-hour light and 12-hour dark cycle. Every 4 days, all seahorses were weighed using their wet mass. This was achieved by gently blotting a seahorse using a paper towel then placing them in a weigh boat sitting on a scale that

had a small amount of salt water in it. Females were visually assessed for cirri presence and candlelit with a flashlight to determine the status of their ovaries. The males collected in the field had to give birth prior to entering the mating trials resulting in 7 trials being arranged on Day 5 of the experiment, and 12 beginning on Day 9 for a total of 19 mating trials (Trials A-S). Each trial consisted of three body weight matched seahorses: one male, one highly ornamented female, and one low or non-ornamented female (Figure 4). The level of cirri ornamentation for the females was assessed qualitatively at the time of trial arrangements but was later verified quantitatively. The males in the trials were observed each day to track when egg transfer occurred and when they gave birth. After the 21 days, any adults that were not assigned into a mating trial were acclimated and released back into Tampa Bay at the sites they were collected in accordance with the SAL protocols. The three adults and offspring born in the trials were sacrificed using MS222 and stored in 70% ethanol for parentage analyses.

Figure 4

Experimental design of the mating trials



One male was paired with one high cirri female and one low cirri female.

New Photography Method for Captive Seahorses

To photograph the female seahorses during this laboratory period, a new protocol was designed to better capture images of the seahorse's cirri. A soft silicon photo stand was constructed using a 15 mL falcon tube as a mold. A small, deep hole was carved into the center

of the flat end of the silicon stand, and then a shallow cut was made through the hole so the stand could be opened and closed to secure a seahorse (Figure 5).

Figure 5

Newly designed photography stand for captive seahorse photos



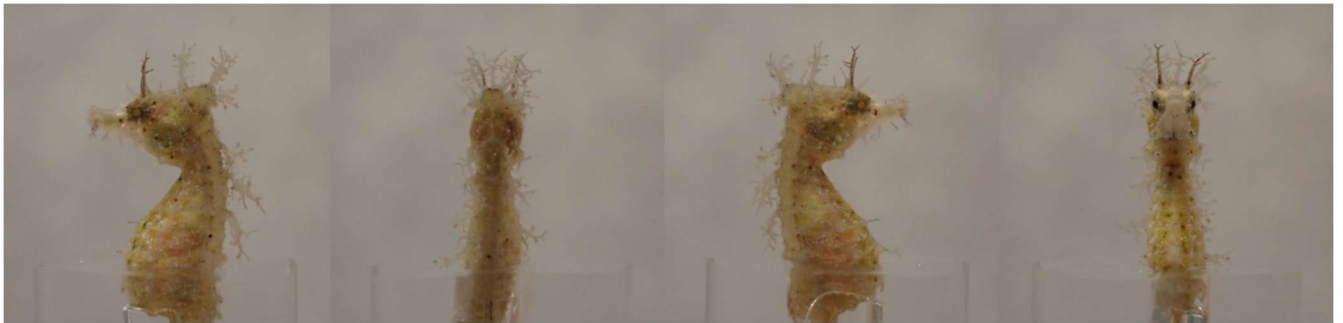
Trial runs of the designed silicon stand were performed using a preserved seahorse.

To allow for easy rotation, the stand was adhered to the center of the outside of a bottom plate of a petri dish. The lid of the petri dish was glued to the bottom of a tank, and the bottom plate could be placed within the lid and slowly spun 360 degrees. Small weights were attached to the inside of the bottom part of the petri dish to weigh it down when submerged in the water. Photos were taken in a 5-gallon tank with white or light grey felt attached to the back to provide a plain background. An Olympus Tough-TG6 camera was used on the microscope setting at 2.8x zoom for photographing. Isolated female seahorses were anesthetized and then gently placed in the silicon stand. The heads and upper bodies of the females were photographed from the left, right, front, and back sides every 4 days while in captivity for a total of 5 documented time points starting with the first day in captivity (Figure 6). Full body photos of the seahorses were also taken by temporarily placing them in a petri dish next to a ruler. Only females were

photographed as preliminary studies showed wild-caught females had more cirri than males, and their cirri presence was more pertinent to the mate choice experiment.

Figure 6

Four angles of female seahorses photographed for laboratory studies



The left, right, front, and back of each seahorse were captured.

Cirri Measurements in Captivity

Protocols were developed to measure cirri number, length, and surface area from the photographs of each female using the program ImageJ (Schneider et al., 2012). Photographs of a metric ruler placed in the silicon stand were taken each photo day, and these photos were used to first set the pixel:mm ratio scale in ImageJ for 2 cm = 20 mm. To measure cirri length, the segmented line tool was used to trace the longest continuous line of the eye, center, and crown cirri from the left side of the seahorse's head. The polygon tool was used to measure the surface area of the cirri at the eye and crown locations on the left side of the seahorse's head using primarily the front and back angle photos. The center cirri were not measured in the surface area protocol due to difficulty in viewing these cirri from the front or back angles and an inability to properly deduce branching from the side angles. Cirri on the left side of the head were measured for length and surface area to maintain consistency with the photos of the seahorses from the year-long sampling. The number of cirri were counted at the eye, center, and crown areas using all four photos of a seahorse. The length measurements were used to quantify the cirri

differences in the females put into the mating trials, as well as the dramatic cirri loss observed in the females over 21 days.

Microsatellite Parentage Analysis

A DNeasy Blood & Tissue Kit (Qiagen Cat. No. 69506) was used to extract DNA from the adults and offspring from each mating trial. Small portions of the tail were dissected from adults, and whole bodies of offspring were used for each extraction. Previously designed *Hzos6* and *Hzos7* primers were utilized and established protocols were followed as described by Rose et al., 2014. The fragment analysis was performed at the Cornell Institute of Biotechnology, and the alleles for the two loci were measured using Peak Scanner Software 1.0 (Applied Biosystems). The parental and offspring alleles were determined for each mating trial to confirm which female mated. A table of all the allele sizes, the number of nucleotides in each amplified genetic fragment, can be found in Appendix C. In 2 of the 19 trials, the males reabsorbed their eggs and did not give birth, so the weights of the females prior and after egg transfer and the visual status of their ovaries post-transfer were used to confidently deduce which female was selected by the male and successfully transferred the eggs.

Statistical Analysis

Confirmation and Determination of the Mating Trials

The total cirri length measurements across the three head areas were calculated for the high-cirri and low-cirri females in the trials to determine if their cirri lengths were significantly different across the two females within each trial. To achieve this, a Shapiro-Wilk normality test was used on the differences between the total cirri lengths of the high and low-cirri females from all the trials, which were determined to be non-parametric. Since trials were set-up on two different days, a test was conducted to determine if there was any potential effect of the number of days since collection on the cirri lengths of the females. A Wilcoxon rank sum test was

performed to compare the cirri length differences between females from the 7 trials set up on Day 5, and the 12 set up on Day 9. The results of this test yielded no significance, signifying that the trials from the two separate days could be combined when analyzing the total cirri lengths of the high and low cirri females. A Wilcoxon signed-rank test was then used to compare the total cirri lengths of the high and low cirri females. The coefficient of variation was used to summarize the similarities between the body weights of the three fish in each trial, but to further analyze the similarities of the body weights, the normality and variance of the differences between the body weights of the paired females in the trials were analyzed using a Shapiro-Wilk test and the variance test function (`var`) in R. The differences were determined to be parametric, so a one-way ANOVA was performed to test if the trials across the two days could be combined. A paired t-test was then conducted on the body weights of the female. A chi-square test was used to determine if there was a significant difference in the number of high and low cirri females chosen as mates.

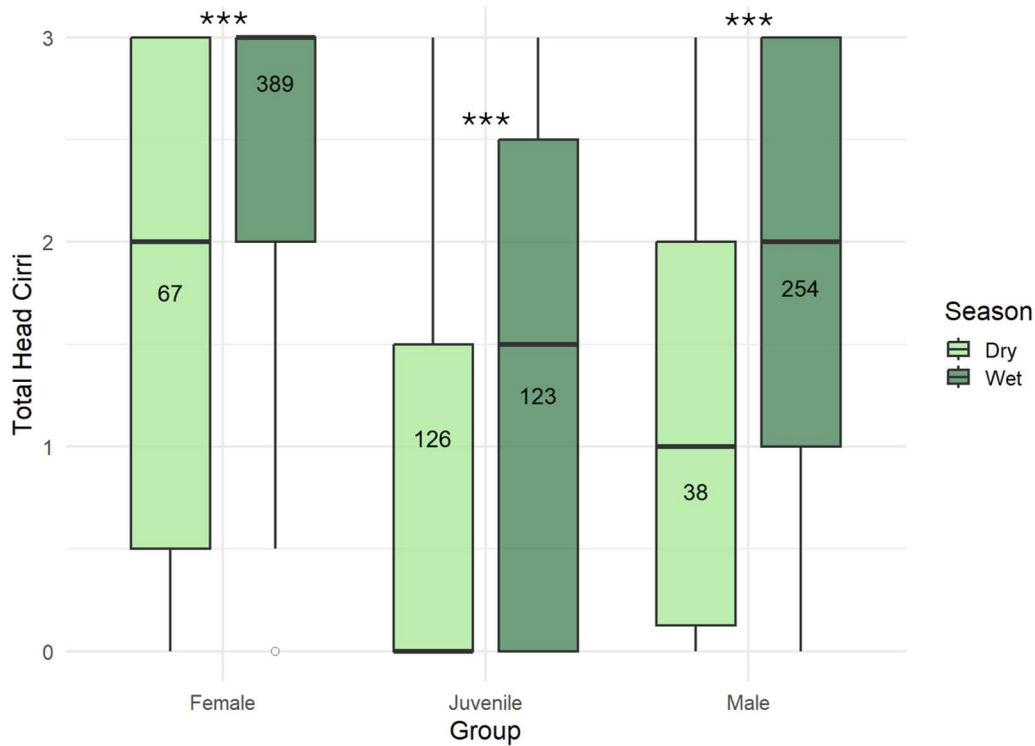
Chapter IV: Results – Wild-Caught Studies

Group and Seasonality Influences on Cirri

When looking at the effects of reproductive group and season on total head cirri, both group ($F_{(2, 991)} = 140.5$, $p < 2e-16$) and season ($F_{(2,991)} = 85.5$, $p < 2e-16$) were found to have significant influences on cirri presence (ART ANOVA; Female: Wet ($n = 389$), Dry ($n = 67$); Male: Wet ($n = 254$), Dry ($n = 38$); Juvenile: Wet ($n = 123$), Dry ($n = 126$)) (Figure 7). Although the overall interaction between group and season was found to be insignificant ($F_{(2,991)} = 0.64$, $p = 0.52$), when looking at the pairwise comparisons, all three groups have significantly different total head cirri between the wet and dry season ($p < 0.0001$) (Figure 7). A full summary of the pairwise comparisons are available in Appendix E. Females had the most cirri of the three groups, and more cirri were present during the wet season for all groups.

Figure 7

Effects of group and seasonality on total head cirri



Pictured is a boxplot showing “Group” (Female, Juvenile, and Male) on the x-axis, and “Total Head Cirri” on the y-axis. The Total Head Cirri are shown for each group between the Wet and Dry seasons. Both group and season were found to have significant effects on cirri presence ($p < 2e-16$), with females having the most cirri, and all groups having more cirri during the wet season. Asterisks indicate significance. The numbers within the boxplots indicate the number of each group caught in each season.

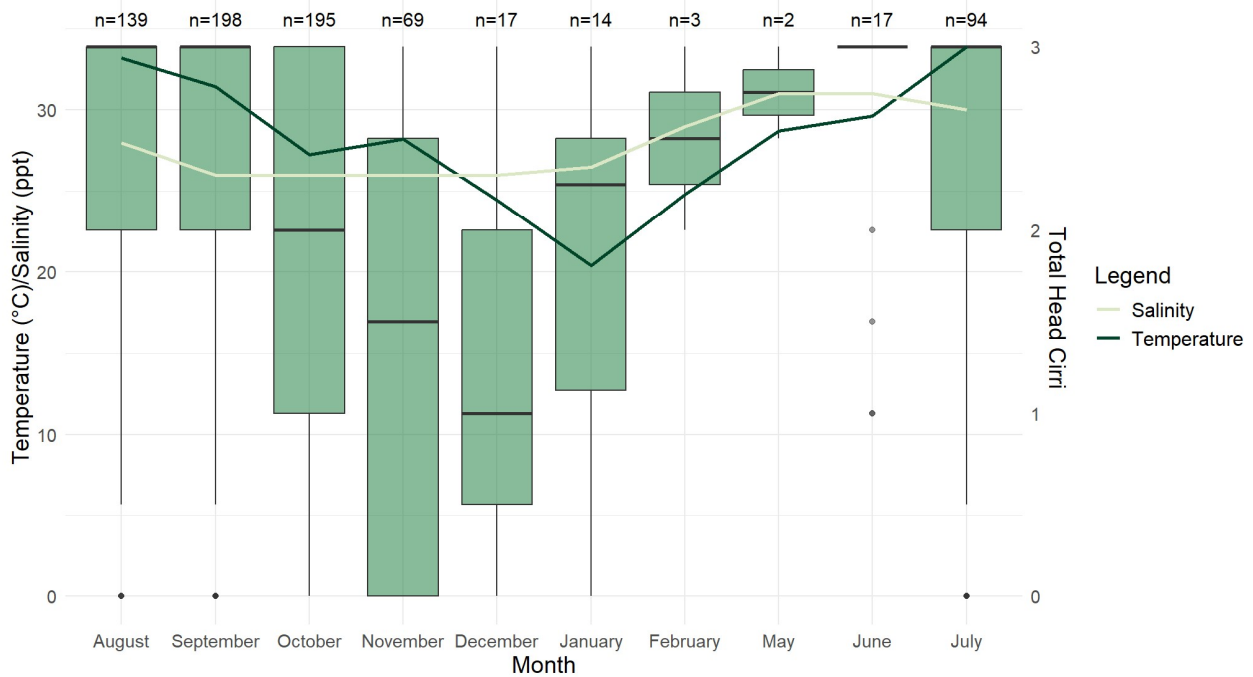
Effects of Abiotic Water Quality Parameters on Cirri Presence

Total head cirri for adult seahorses were found to significantly increase with the main effects of salinity (GLM, 0.518 ± 0.171 , $t = 3.03$, $p = 0.00255$, $n = 748$) and temperature (GLM, 0.404 ± 0.144 , $t = 2.80$, $p = 0.00529$, $n = 748$) (Figure 8) (Table 1). Additionally, a significant interaction was seen between temperature and salinity (GLM, -0.0148 ± 0.00539 , $t = -0.015$, $p =$

0.0060, n = 748) (Table 1). This interaction had a negative t-value though, indicating that the effects of temperature on total head cirri may decrease as salinity increases. A full summary of this model is available in Table 1.

Figure 8

Effects of temperature and salinity on total head cirri



Pictured is a combined boxplot and line graph depicting how adult “Total Head Cirri,” “Temperature,” and “Salinity” fluctuate over time. Sampling “Months” are displayed on the x-axis, while the right-hand y-axis reports Total Head Cirri seen in the boxplots, and the y-axis on the left side coincides with the lines for Temperature and Salinity. A general trend can be seen across the three variables, with all of them decreasing in the colder months, and increasing for warmer months. GLM analysis confirmed that Salinity and Temperature have significant effects on Total Head Cirri ($p < 0.05$). N values are the number of adult seahorses caught each month.

Table 1*Summary statistics of the abiotic water quality GLM*

GLM (Gamma)	Estimate	Standard Error	t value	p value
Intercept	-13.41	4.587	-2.923	0.00358
Temperature	0.4038	0.1444	2.797	0.00529
Salinity	0.5189	0.1714	3.027	0.00255
SexMale	-0.3012	0.03444	-8.744	< 2e-16
SeasonWet	0.2928	0.05579	5.249	2e-07
Temp:Salinity	-0.01478	0.005368	-2.754	0.00603

All predictor variables and the interaction between temperature and salinity were determined to be significant. The intercept represents SexFemale and SeasonDry.

Effects of Macroalgae on Cirri Presence

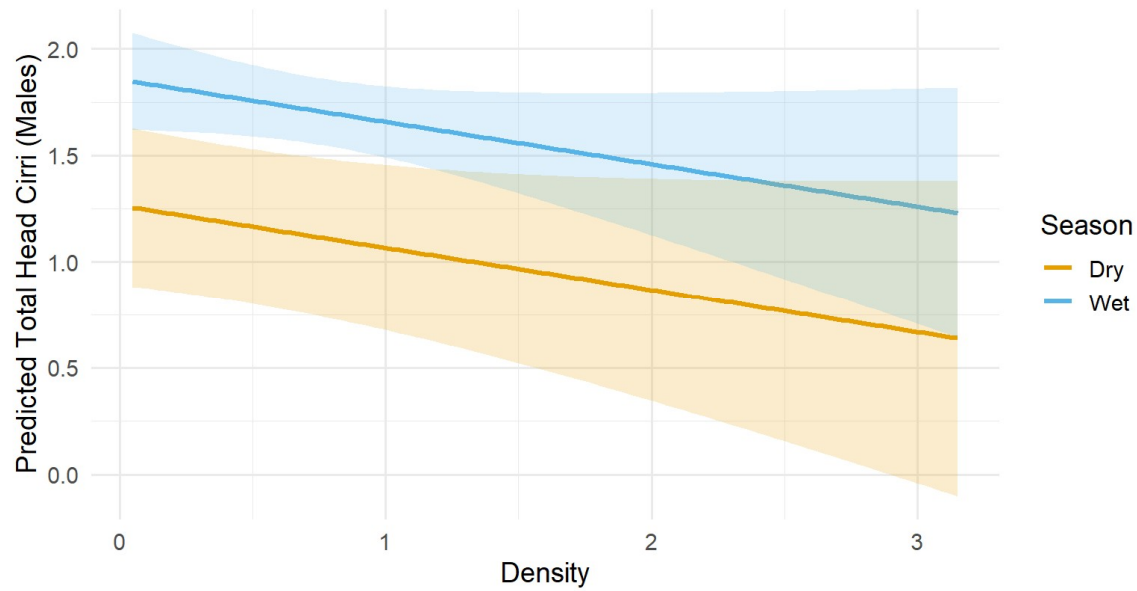
Macroalgae density was found to not have a significant effect on total head cirri (GLM, 0.0519 ± 0.0297 , $t = 1.75$, $p = 0.0807$, $n = 561$).

Effects of Population Factors on Cirri Presence

Total density was found to have a significant effect on head cirri presence (GLM, -0.172 ± 0.0614 , $t = -2.800$, $p = 0.029$, $n = 609$). Density was found to have a negative effect on head cirri for both males (Figure 9) (Table 2) and females (Figure 10) (Table 2), meaning that total head cirri scores decreased in high density areas. The results of the full model can be found in Table 2.

Figure 9

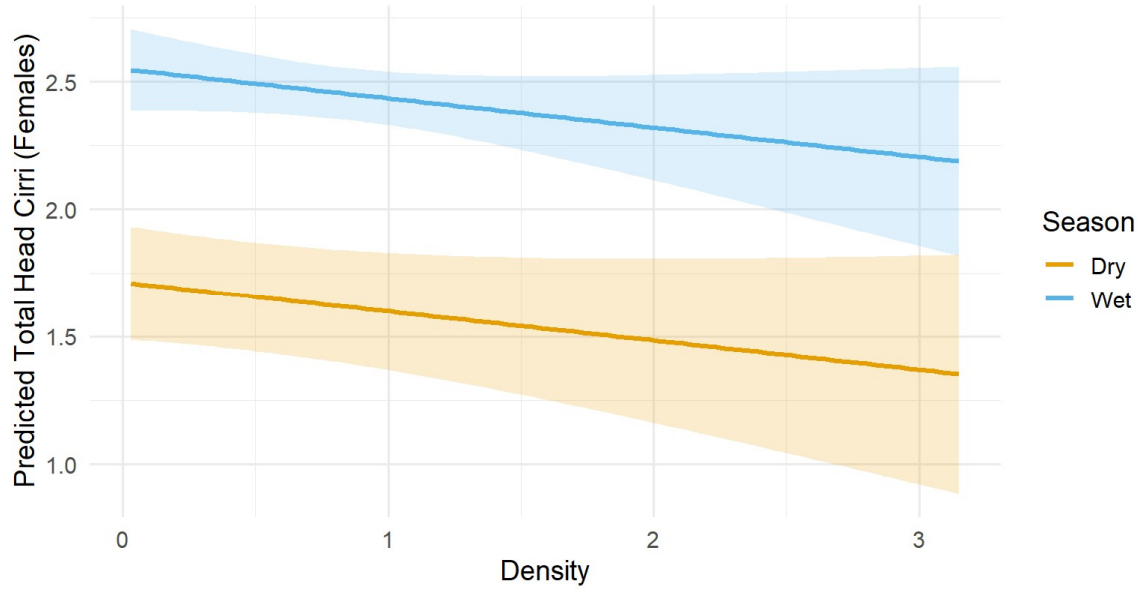
Effects of density on total head cirri (males)



The line graph depicts “Predicted Total Head Cirri” for males over “Density” (total number of seahorses/m² per transect) in the wet and dry seasons. A decrease in total head cirri is seen as densities increase.

Figure 10

Effects of density on total head cirri (females)



The line graph depicts “Predicted total head cirri” for females over “Density” (total number of seahorses/m² per transect) in the wet and dry seasons. A decrease in total head cirri is observed as densities increase.

Table 2

Summary statistics of the population factors GLM

GLM (Gaussian)	Estimate	Standard Error	t value	p value
Intercept	1.811	0.1063	17.04	< 2e-16
SexMale	-0.7128	0.1063	17.04	< 2e-16
SeasonWet	0.7471	0.1102	6.782	2.82e-11
Density	-0.1475	0.06741	-2.189	0.029

All predictor variables were significant. Density was the focus of this model and was found to have a negative effect on total head cirri. The intercept represents SexFemale and SeasonDry.

Chapter V: Results – Laboratory Studies

Cirri Preference in Males

To confirm the mating trials were set-up accurately, the coefficient of variation (CV) between the body weights of the three fish put in each trial was calculated. All the trials had a CV of less than 10%, with most of the trials being below 5% (Table 3). In the few trials with a higher CV%, this was due to the male's body weight being slightly deviated from the females' weights. The weights of the females had no significant differences, confirming that the males were still choosing between females matched for their body weights in each trial (Paired t-test, $t_{(18)}, p = 0.6311$) (Figure 11). The total cirri lengths of the females within each of the trials were compared and determined to be significantly different across the paired females, thus quantitatively confirming the qualitative pairings for each replicate (Wilcoxon signed-rank test, $n = 38, p = 3.815e-06$).

Table 3

Summary table of the body weights for the three fish in each trial.

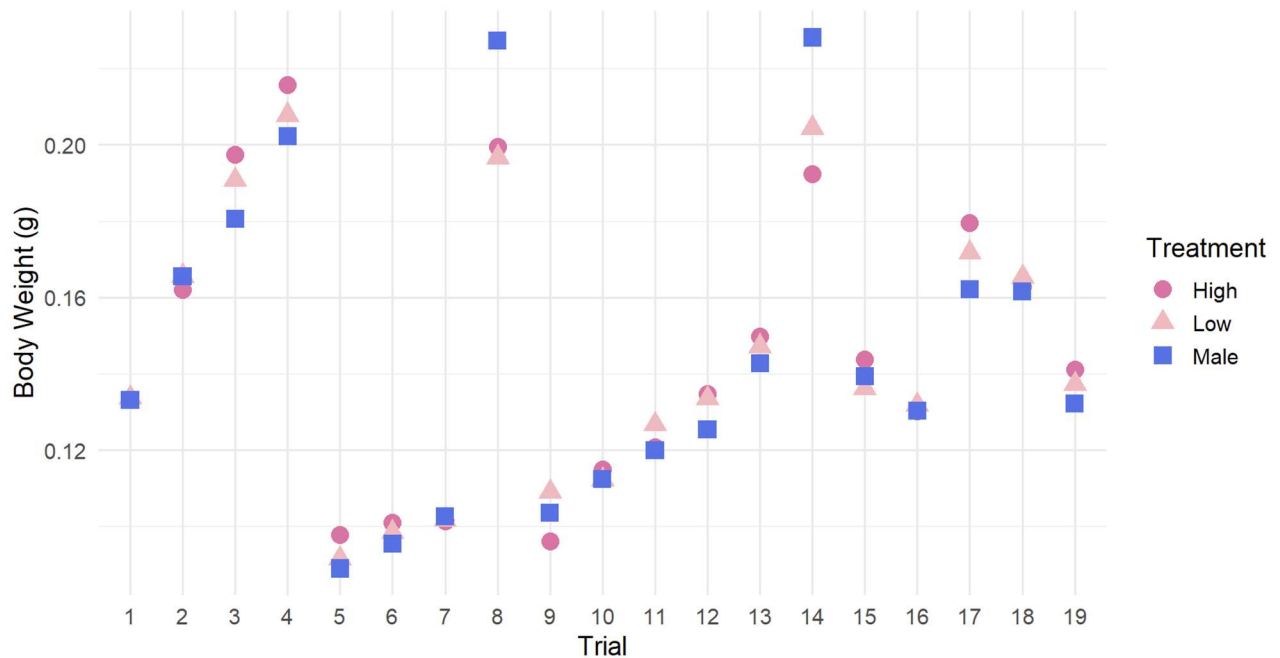
Replication	n	Min	Max	Range	Mean	SD	CV%
1	3	0.133	0.134	0.000600	0.133	0.000321	0.241
2	3	0.162	0.166	0.00350	0.164	0.00199	1.21
3	3	0.181	0.198	0.0169	0.190	0.00851	4.49
4	3	0.202	0.216	0.0134	0.209	0.00674	3.23
5	3	0.089	0.0978	0.0088	0.0928	0.00453	4.89
6	3	0.0955	0.101	0.00550	0.0983	0.00275	2.80
7	3	0.101	0.103	0.00120	0.102	0.000624	0.613
8	3	0.197	0.227	0.0305	0.208	0.0169	8.11
9	3	0.0961	0.109	0.0130	0.103	0.00653	6.34
10	3	0.112	0.115	0.00280	0.113	0.00154	1.36

11	3	0.12	0.127	0.00670	0.122	0.00366	2.99
12	3	0.125	0.135	0.00940	0.131	0.00509	3.88
13	3	0.143	0.150	0.00690	0.147	0.00349	2.38
14	3	0.192	0.228	0.0357	0.208	0.0182	8.72
15	3	0.136	0.144	0.00760	0.140	0.00382	2.73
16	3	0.130	0.132	0.00160	0.131	0.000896	0.685
17	3	0.162	0.180	0.0174	0.171	0.00872	5.09
18	3	0.162	0.165	0.00390	0.163	0.00199	1.22
19	3	0.132	0.141	0.00900	0.137	0.00451	3.30

All trial body weights had a CV% of less than 10%, with most of the trials being below 5%.

Figure 11

Visualization of the body weights of the three fish put in each trial



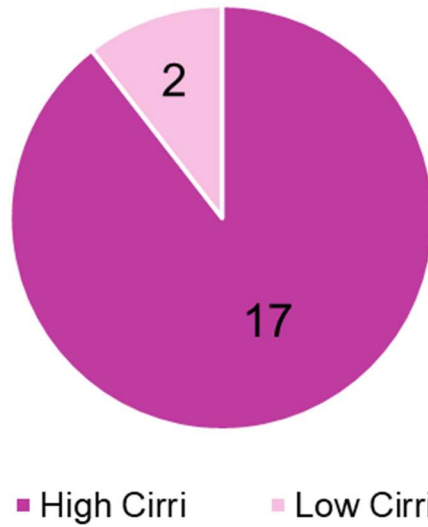
The weight of the male was distant in a few of the trials, but the females in every trial had similar weights (Paired t-test, $t_{(18)}$, $p = 0.6311$).

After confirmation through microsatellite parentage analysis, as well as the alternative methods used for the two trials where the males did not give birth, males chose to mate with the high cirri female in 17 of the 19 trials. A significant preference is exhibited by the males,

although the motive behind this preference is unknown (Chi-square, High cirri n = 17, Low cirri n = 2, df = 1, $X^2 = 13.47$, $p < 0.05$) (Figure 12).

Figure 12

The distribution of high and low cirri females chosen in the mating trials



High cirri females were chosen in 17 out of 19 trials, exhibiting a strong male preference for high cirri females (Chi-square, $p < 0.05$).

Chapter VI: Discussion

The central hypothesis of this study, that cirri in *H. zosterae* may function as a sexually selected, condition-dependent trait, was first supported by several patterns observed in wild-caught dwarf seahorses. In the surveys of the natural populations of dwarf seahorses in this study, seasonal cirri patterns across all three demographic groups in *H. zosterae* were discovered that align with patterns reported in other seahorse species. Female *H. zosterae* had more cirri than the males, similar to a trend reported in *H. guttulatus* (Woodall et al., 2018). Juvenile *H. zosterae* had substantial amounts of cirri, although less than adults, indicating cirri development does not coincide with the onset of sexual maturity in this species, which is observed in *H. guttulatus* as well (Curtis, 2006). Cirri presence in *H. zosterae* does not seem to occur randomly as reported in *H. hippocampus*, though, as we can confidently predict more cirri to be present in sexually mature adults (Curtis, 2006; Curtis & Vincent, 2005).

In addition to females having more cirri than males, which is indicative of a sexually dimorphic trait in *H. zosterae*, cirri were found to be a seasonal trait. More cirri were observed in seahorses caught during the wet season, which coincides with the peak breeding season of dwarf seahorses. These two results of sexual dimorphism and seasonality of cirri presence led to the hypothesis that cirri might be a sexually selected trait in *H. zosterae*.

The laboratory mate preference trials were conducted to test the hypothesis that cirri are functioning as a secondary sex trait in females. From these trials, we found that the males exhibited a strong preference for high cirri females, with the highly ornamented female being

chosen as a mate in 17 of the 19 trials. The quantity and size range availability of wild caught individuals were limited, particularly for male seahorses. Collection samplings were only performed over two days to control the timeframe when mating trials were conducted, which led to a few of the trials having a male more distant in weight from the females. However, even in the trials with higher CV% values, the males were still choosing between two body weight size-matched females to exclude any existing preferences for body size when selecting a mate. Interestingly, the two trials where the low cirri female was chosen (Trials 8 and 14) had the highest CV%, and the males from these trials were also the largest ones used in the study.

The results from the laboratory study are promising, as they provide support for cirri in seahorses having a function pertaining to mating, but more mating trials should be conducted to investigate other factors that could have influenced male mate choice. For example, many species of Syngnathids, including *H. zosterae*, execute elaborate courtship behaviors prior to copulation (Berglund et al., 1986; Berglund et al., 1997; Christie, 2022; Masonjones & Lewis, 1996). Courtship behaviors are ritually performed before mating but are influenced by density in a laboratory setting (Masonjones & Rose, 2019). Courtship behaviors are exhibited more frequently in high density tanks, but mating success is decreased when multiple males are present due to aggressive actions towards other males (Masonjones & Rose, 2019).

Understanding how densities may affect mating success in dwarf seahorses could provide some rationale behind the negative relationship found between cirri presence and density in wild-caught seahorses. While male dwarf seahorses typically perform a greater number of behaviors than the females and are more likely to initiate certain behaviors, females who are more active and receptive of the male's courtship have better chances of successfully mating (Masonjones & Lewis, 1996; Patel, 2025). Behaviors were not recorded for the mating trials in this study, but

further observations of female activity and the correlation between mating effort with cirri presence could reveal potential relationships between the two factors. In the instance that females with more cirri exhibit greater levels of courtship behaviors, this could support the notion of females with more cirri having higher energy resources and thus being of better quality and preferred as mates.

Chemical cue signaling is not well-studied in syngnathids, but in the Gulf pipefish (*Syngnathus scovelli*), which inhabit the same seagrass beds as dwarf seahorses in Tampa Bay, males detect and show a preference for chemical cues from females (Ratterman et al., 2009). Olfactory cues are also crucial for females in *Hippocampus erectus*, as females use these cues to recognize their mates (Lin et al., 2021). The utilization of chemical cues in *H. zosterae* is unknown but given the possibility that females could use chemical cues to communicate with males, high quality females that potentially have more cirri may produce different signals than those of lower quality. If females with more cirri are of higher quality and can effectively communicate that via a signal to males through olfactory cues, this could be another factor influencing male mate choice.

Along with the mate preference trials, another important component to the laboratory studies was the documentation of cirri loss in females throughout the 21 days of captivity. The photos of wild-caught seahorses were originally taken for facial recognition software, rather than for cirri measurements, prompting the development of a novel photographing method for the laboratory portion for repeated monitoring of the captive seahorses in the study. Through these photos, drastic loss of cirri pigmentation, length, and branching, was observed for all the females in captivity (Appendix D). Although the length and surface area measurements of the cirri are still in progress, preliminary results for cirri lengths have confirmed these loss trends

quantitatively (Appendix D). While not tested in *H. zosterae*, cirri that have been cut can be regenerated in other species of seahorses, even within a laboratory setting (Correia et al., 2018; Gristina et al., 2017). The loss of cirri during captivity and lack of development in captive raised offspring with limited food resources in dwarf seahorses, along with evidence of the ability for other species to regrow cirri, suggests this trait's potential as a phenotypically plastic, condition-dependent trait.

Sexually selected and condition-dependent ornamental traits are well-documented in the Syngnathidae family. While none are reported in seahorses, females in several species of pipefish exhibit plastic, condition-dependent ornamental features that are thought to be under sexual selection pressures (Berglund et al., 1997; Mobley et al., 2018; Monteiro et al., 2017; Rosenqvist & Berglund, 2011; Takahashi et al., 2003). In these unique species with male mate choice, female ornamentation is unlikely to be detrimental to the females and often aids in attracting a mate (Schlupp, 2018). Male pipefish appear to prefer females with more ornamentation, as these traits can be honest signals of the females' reproductive quality, with more ornamented females having direct mating benefits such as higher fecundity or larger eggs (Berglund et al., 1997; Mobley et al., 2018; Monteiro et al., 2017). The males' preference for ornamentation drives sexual selection pressures on females (Rosenqvist & Berglund, 2011; Schlupp, 2018). Sexual selection pressures are generally stronger in species that exhibit polyandrous, polygamous, or promiscuous mating systems, which often occur in pipefish species, but can also influence monogamous systems (Rosenqvist & Berglund, 2011). For example, in polyandrous *S. scovelli*, females have been shown to have iridescent bands on their torso that vary across geographic populations, are induced in males during exposure to synthetic estrogen (EE2), and are sexually selected with females possessing greater and larger bands

resulting in higher mating and reproductive success (Flanagan et al., 2014; Tosto et al., 2023). In the monogamous species *Hippocampus subelongatus*, females were under stronger sexual selection pressures than the males due to male preference for larger body size, and competition due to a female biased sex-ratio (Rosenqvist & Berglund, 2011). Despite dwarf seahorses' monogamous mating system, sexual selection may still be acting on cirri presence through male preference for this trait.

The reasons behind this preference are still unknown, but like other members of the Syngnathidae family, cirri may be an ornamental trait that is an honest signal of female quality (Berglund et al., 1997; Mobley et al., 2018; Monteiro et al., 2017). Further experimentation would need to be conducted to determine cirri's role as an honest signal in seahorses, but there is a possibility that females with more cirri could have direct mating benefits that drive the preference shown in the mating trials. Future studies looking at egg yolk or fat content in female seahorses could provide evidence for cirri as an honest signal. Egg quality in fishes is highly variable, with a multitude of factors such as nutrients in the oocytes, female endocrine status, and the conditions of the surrounding water influencing quality, but females who exhibit more extravagant ornamentation may be using it to signal their quality to males or using the cirri as energy reserves (Brooks et al., 1997).

Besides the sexual selection influences on cirri, other factors related to the environment of the seahorse could contribute to its condition-dependency. While being held in captive settings, seahorses are often lacking a variety of environmental influences, such as predator cues and other chemical signals from their typical surrounding habitat. If cirri contribute to a seahorse's camouflage due to natural selection pressures, a sudden lack of cues from their natural ecosystem could cause a decrease of energy and effort towards maintaining their cirri. In a

laboratory setting, they are also being fed a different, potentially more nutrient-limited diet than what they would normally consume in the wild. Brine shrimp allows researchers to provide the seahorses with a consistent food source in the lab, but as a single food source, brine shrimp have been shown to be an inadequate nutritional profile for seahorses (Randazzo et al., 2018). Likely, a combination of the missing environmental cues and diet contributes to the decline in cirri maintenance observed during the laboratory studies. The wild-caught studies suggested that temperature and salinity had positive effects on cirri presence, with more cirri being present in months with higher temperatures and salinities, and although macroalgae densities had no effect on cirri presence, this result could change depending on the type of macroalgae sampled. These environmental variables can also be correlated to seahorses' diet in the wild, as plankton, a common food source for seahorses, are more abundant when temperatures and salinities are higher (Badylak et al., 2007).

The documented loss of cirri phenomenon leads to even more interesting future directions, as a multitude of studies could be conducted to investigate the hypothesis of condition-dependency for cirri. Seahorses kept in a laboratory setting could be provided with a variety of food sources with different nutritional values or be exposed to predator chemical cues and photographed over time to see how these conditions affect the presence and level of ornamentation of their cirri. Looking further into food sources is an important future direction, as copepods are vital for the early development stages of multiple types of seahorses, and foods that are richer in carotenoids may influence their ability to maintain their coloration (Randazzo et al., 2018; Segade et al., 2015). Given that the laboratory seahorses in this study substantially lost cirri pigmentation, a more robust diet may allow seahorses in captivity to retain their cirri coloration and ornamentation levels for a longer duration of time. Different kinds of

environmental parameters in the wild can also be measured, such as densities of multiple types of submerged aquatic vegetation, varying species of macroalgae, seagrass blade heights, and epiphytic growth on seagrass to explore how these factors affect cirri presence.

While this project led to many novel findings, there were some limitations in the experimental designs to be considered for future studies, particularly for the wild-caught portion. Due to the brief amount of time that wild-caught seahorses were handled, the photographs of the wild seahorses do not capture cirri in an optimal fashion. These seahorses were only photographed on their left side, not allowing for proper viewing of the cirri branching, and because the seahorses were held against the side of the tank, the cirri were at times folded over and not displayed properly. The combination of these factors can create challenges when attempting to determine exact measurements of cirri when using the photos of the wild-caught. Additionally, the sampling event during August in this study was the first official sampling conducted for the wild-caught seahorses and was recorded more simply compared to the rest of the collecting events, leading to less detailed information for each transect. As a result of this, all seahorses were grouped only by site, north or south, and there is no transect level data for the August sampling, lowering the number of seahorses represented in the wet season for the biotic and population factors analyses. Certain issues when recording environmental parameters also occurred throughout the year-long sampling period. Percent seagrass cover was not recorded throughout the entire year, preventing this factor from being included in the analyses for this study. The salinity and temperature measurements are also for nearby data collection buoys further from shore in the bay instead of directly at the shallow described sampling sites, as these measurements were recorded from the National Oceanic and Atmospheric Administration (NOAA) buoys closest to the collection sites.

New information about cirri in *H. zosterae* and seahorses overall has been suggested through the findings of this thesis. These results suggest that some biological and environmental factors, such as sex, season, temperature, salinity, and population densities can possibly predict cirri patterns in wild-caught dwarf seahorses. Through the laboratory studies, cirri's potential as a sexually preferred trait in *H. zosterae* was investigated and established. Additionally, when looking at cirri loss in captivity, the dramatic decline in cirri in a laboratory setting could imply the use of cirri as an indicator of seahorse health. Being able to visually assess seahorse health through physical traits such as cirri have important implications, especially since dwarf seahorses are indicator species for the vital seagrass beds they inhabit (Shokri et al., 2009). The seagrass beds in Tampa Bay have experienced both substantial declines and increases over the years, and learning more about the health of the dwarf seahorses subsequently allows researchers to learn more about the health of the seagrass beds (Sherwood et al., 2017). While there are many future directions to explore cirri further, this thesis provides the groundwork for more thorough investigations into this novel trait exhibited by many seahorse species, and further implications about the importance of cirri and seahorses within their ecosystem.

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Appendix A:
IACUC Approval for Wild-Caught Studies



VALDOSTA STATE UNIVERSITY

Institutional Animal Care and Use Committee (IACUC)
Animal Use Protocol Approval

June 17, 2021

Dr. Emily Rose
Department of Biology
Valdosta State University

Dear Dr. Rose;

Animal Use Protocol (AUP) *“The effects of water turbidity on seahorse mating behaviors and reproduction.”* (AUP-00081-2021) has been approved by the Institutional Animal Care and Use Committee (IACUC). This approval is from 06.17.2021 – 06.17.2024. Each year, an animal report must be submitted to the IACUC to keep your protocol active. You will be contacted by the Office of Sponsored Programs and Research Administration approximately one month before the annual report is due.

Please remember that you must obtain IACUC approval before amending, or altering the scope, or procedures of the protocol. You are also required to report to attending Veterinarian, the IACUC Chair, and the IACUC Administrator any unanticipated problems with the animals that become apparent during the course, or as a result of the research, or teaching activity.

Should you have questions concerning your approved research, please contact Tina Wright, Compliance Specialist, 229.253.2947, email tmwright@valdosta.edu, or IACUC Alias @ iacuc@valdosta.edu.

Sincerely,

Elizabeth W. Olphie
Elizabeth “Ann” Olphie
IACUC Administrator

OFFICE of SPONSORED PROGRAMS & RESEARCH ADMINISTRATION (OSPRA)
PHONE 229.249.2614 • FAX 229.245.3853 • WEB www.valdosta.edu/ospra/
ADDRESS 1500 N. Patterson St. • Valdosta, GA 31698 • LOCATION Room 3100 • Converse Hall
A Comprehensive University of the University System of Georgia and an Equal Opportunity Institution

Appendix B:
IACUC Approval for Laboratory Studies



Institutional Animal Care and Use Committee (IACUC)

ANIMAL USE PROTOCOL APPROVAL

June 14, 2024

Dr. Emily Rose
Department of Biology
Valdosta State University

Dear Dr. Rose;

Animal Use Protocol (AUP) "*Spatial Analysis of the "Investigating the retention of cryptic filamentous appendages in seahorses"*" (AUP-00086-2024) has been approved by the Institutional Animal Care and Use Committee (IACUC). AUP approval is from 06.14.2024 – 06.14.2027. Each year, an animal use report must be submitted to the IACUC to keep your protocol active. You will be contacted by the Office of Sponsored Programs and Research Administration approximately one month before the annual report is due.

Please remember that you must obtain IACUC approval before amending, or altering the scope, or procedures of the protocol. You are also required to report to attending Veterinarian, the IACUC Chair, and the IACUC Administrator any unanticipated problems with the animals that become apparent during the course, or as a result of the research, or teaching activity.

Should you have questions concerning your approved research, please contact Tina Wright, Compliance Officer, at 229.253.2947, or email tmwright@valdosta.edu.

Sincerely,

Ann

Elizabeth "Ann" Olphie
IACUC Administrator

cc: Dr. Becky da Cruz, Associate Provost for Graduate Studies and Research
Dr. Teresa Doscher, Attending Veterinarian
Dr. Robert L. Gannon, Department Head

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Appendix C:
Allele Calls for Parentage Analysis

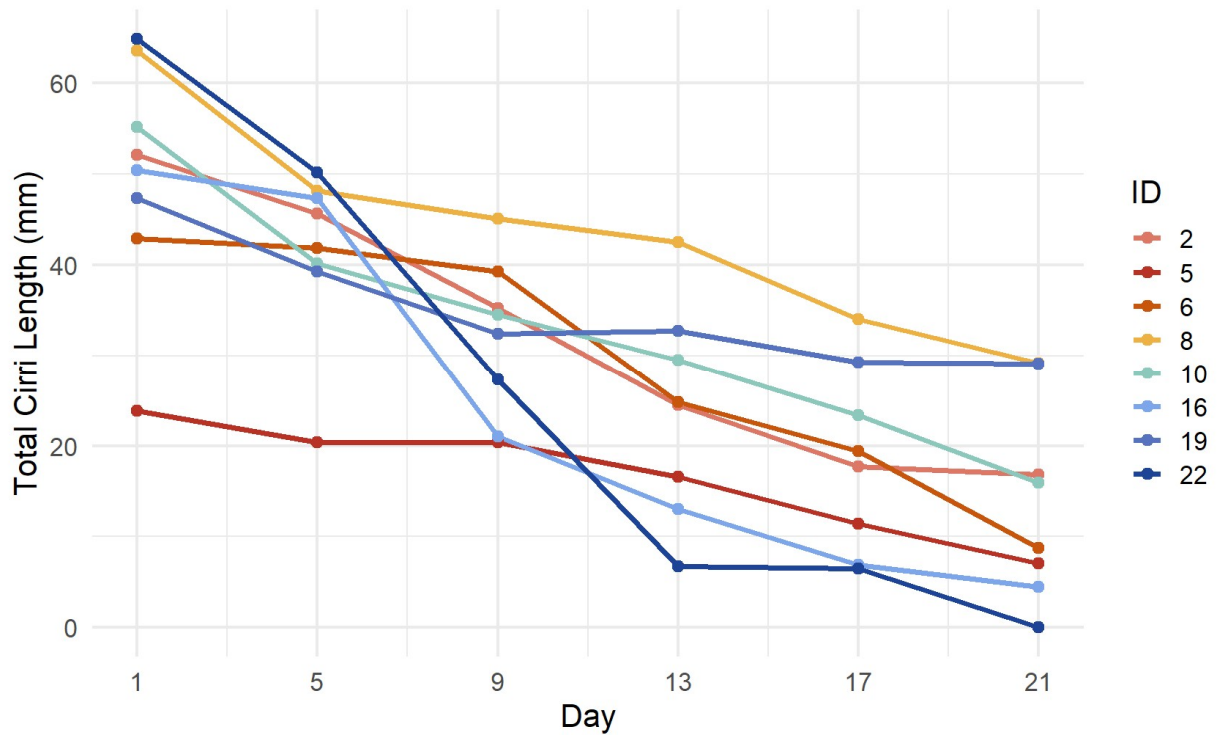
Appendix C. Allele calls for every mating trial for the parentage analysis. All alleles that were amplified and confidently scored using Peak Scanner are reported.

ID	07		06	
	Allele 1	Allele 2	Allele 1	Allele 2
A F1	172	172	280	303
A F2	232	245	303	311
AB1	232	232	311	356
AB2	245	252	311	360
AB3	232	232	311	356
A MALE	252	232	356	360
B F3	256	283	332	372
B F6	228	256	328	328
BB1	216	228	328	328
BB2	216	256	328	328
BB4	NA	NA	309	328
B MALE	203	216	309	328
C F7	244	252	273	320
C F8	175	279	316	403
CB1	175	224	387	403
CB2	228	279	316	387
CB3	175	224	316	387
C MALE	224	228	265	387
D F9	220	236	277	280
D F10	232	268	292	316
DB1	220	232	316	325
DB2	244	268	292	325
DB3	244	268	316	325
D MALE	220	244	325	325
F F17	264	294	372	372
F F14	248	258	300	307
FB1	240	258	300	300
FB2	216	248	307	307
FB3	240	258	307	360
F MALE	216	240	307	360
G F37	249	298	265	360
G F40	208	236	280	336
GB1	216	236	278	336
GB2	208	216	280	296

G MALE	216	256	278	296
H F41	282	282	288	345
H F42	248	252	258	296
HB1	282	294	288	324
HB2	282	294	288	324
H MALE	287	294	161	324
J F49	253	264	269	325
J F24	248	271	303	395
JB1	240	248	353	395
JB2	223	248	314	395
J MALE	223	240	314	353
K F39	256	268	320	345
K F18	245	249	353	391
KB1	249	260	320	353
KB2	208	245	320	391
K MALE	208	260	320	320
L F27	216	335	161	324
L F20	232	272	161	299
LB1	232	306	161	320
LB2	272	306	299	320
L MALE	271	306	320	320
M F51	211	253	254	336
M F30	252	252	277	285
MB1	267	267	285	307
MB2	267	267	277	307
MB4	252	252	277	307
M MALE	252	267	376	307
N F31	236	290	261	301
N F36	264	264	292	408
NB1	252	290	261	301
NB2	264	290	301	301
N MALE	252	264	301	320
O F32	232	253	296	296
O F48	244	275	320	330
OB1	219	275	320	320
OB2	219	275	320	320
O MALE	219	223	292	320
P F33	244	306	314	314
P F26	264	275	332	336
PB1	240	275	332	368

PB2	240	275	332	364
P MALE	240	268	364	368
Q F21	224	260	273	281
Q F44	232	244	254	320
QB1	232	244	320	345
QB2	244	244	254	376
Q MALE	211	244	345	376
R F23	264	314	246	273
R F29	228	248	334	334
RB1	199	314	246	332
RB2	264	279	246	285
R MALE	199	279	285	332
S F15	228	248	321	321
S F38	245	287	300	300
SB1	216	287		300
SB2	216	245	277	300
S MALE	216	236	277	277

Appendix D:
Cirri Loss Trends in Captive Female Seahorses



Appendix D. Photos of the same female seahorse on Day 1, Day 5, Day 13, and Day 17, and a visualization of cirri length loss over time. Dramatic loss of cirri pigmentation, length, and branching can be seen over time in the photos. A line graph was made to visualize the decline in total cirri length throughout the entire 21 days in captivity for multiple females

Appendix E:
Pairwise Comparisons for Sex and Season

Appendix E. Full summary of significant pairwise comparisons between the six combinations of group and season. Bolded values are those represented in Figure 8 by asterisks.

Contrast	Estimate	Standard Error	t ratio	p value
Female Dry – Juvenile Dry	0.9986	0.0829	12.050	< 0.0001
Female Dry - Male Dry	0.6658	0.0740	9.002	< 0.0001
Female Dry - Female Wet	-0.7599	0.0799	-9.510	< 0.0001
Female Dry - Juvenile Wet	0.2387	0.1340	1.787	0.4744
Female Dry - Male Wet	-0.0941	0.1080	-0.872	0.9530
Juvenile Dry - Male Dry	-0.3328	0.0903	-3.688	0.0032
Juvenile Dry - Female Wet	-1.7585	0.0931	-18.889	< 0.0001
Juvenile Dry - Juvenile Wet	-0.7599	0.0799	-9.510	< 0.0001
Juvenile Dry - Male Wet	-1.0927	0.0986	-11.078	< 0.0001
Male Dry - Female Wet	-1.4257	0.1100	-12.977	< 0.0001
Male Dry - Juvenile Wet	-0.4271	0.1390	-3.072	0.0265
Male Dry - Male Wet	-0.7599	0.0799	-9.510	< 0.0001
Female Wet - Juvenile Wet	0.9986	0.0829	12.050	< 0.0001
Female Wet - Male Wet	0.6658	0.0740	9.002	< 0.0001
Juvenile Wet - Male Wet	-0.3328	0.0903	-3.688	0.0032